

Europäisches Patentamt

European Patent Office

Office européen des brevets



(11)

**EP 1 018 549 A1**

(12)

**EUROPEAN PATENT APPLICATION**

published in accordance with Art. 158(3) EPC

(43) Date of publication:

**12.07.2000 Bulletin 2000/28**

(51) Int. Cl.<sup>7</sup>: **C12N 15/10**

(21) Application number: **98943033.5**

(86) International application number:

**PCT/JP98/04201**

(22) Date of filing: **18.09.1998**

(87) International publication number:

**WO 99/15645 (01.04.1999 Gazette 1999/13)**

(84) Designated Contracting States:

**CH DE FR GB LI SE**

(30) Priority: **22.09.1997 JP 25679597**

(71) Applicants:

• **THE INSTITUTE OF PHYSICAL & CHEMICAL RESEARCH**

**Wako-shi, Saitama 351-0198 (JP)**

• **Hayashizaki, Yoshihide**

**Tsukuba-shi, Ibaraki 305-0074 (JP)**

(72) Inventors:

• **HAYASHIZAKI, Yoshihide**

**Inst.Phys./Chem.Res.Tsukuba**

**Ibaraki 305-0074 (JP)**

• **CARNINCI, Piero-Inst. of Phys./Chem. Res.**

**Tsukuba**

**Ibaraki 305-0074 (JP)**

(74) Representative:

**Godemeyer, Thomas, Dr.**

**Sternagel, Fielscher, Godemeyer & Partner**

**Patentanwälte**

**An den Gärten 7**

**51491 Overath (DE)**

(54) **METHOD FOR ISOLATING DNA**

(57) A method for isolating DNA comprising: (A) feeding into a column provided with a DNA-binding carrier on a membrane filter a solution which contains a biological sample containing a salt, a cationic surfactant and a DNA and in which the concentration of the salt is not lower than the precipitation inhibition starting concentration and thus making the DNA contained in the biological sample to bind to the DNA-binding carrier; (B) sucking off the solution from the column and separating the carrier having the DNA bound thereto from other components; (C) feeding a DNA-dissociating solution into the column to thereby dissociate the DNA from the carrier; and (D) sucking off the dissociation solution from the column and taking up the solution containing the dissociated DNA. According to this method, a purified DNA can be taken up in a high yield without resort to any pretreatment of a biological sample.

**EP 1 018 549 A1**

## Descripti n

### Technical Field

[0001] The present invention relates to a method for isolating DNA comprised in biological samples such as blood, cells and biological tissues.

### Background of the Invention

[0002] Transformation of microorganisms such as *Escherichia coli* and the like, the culturing of the resulting transformant, and the recovery of a desired plasmid DNA from the proliferating transformant have been carried out routinely in the field of genetic engineering. For the purpose of collecting DNA information concerning cancer and genetic diseases and using the DNA information for diagnosis, additionally, DNAs comprised in biological samples such as blood, cells and biological tissues are recovered.

[0003] The method for more simply recovering and purifying plasmid DNAs from transformants includes, for example, methods described in Japanese Patent Laid-open No. 360686/1992, Japanese Patent Laid-open No. 23976/1996, R. Room et al., J. Clin. MicroBiol. Vol. 28, No. 3, p. 495-503, and Japanese Patent Laid-open No. 250681/1995.

[0004] Among them, the chaotropic ion method described in R. Room et al., J. Clin. MicroBiol. Vol. 28, No. 3, p. 495-503 is an excellent method for isolating DNA alone, comprising separating RNA and DNA comprised in microorganisms from each other by using a DNA-adsorbing carrier and a chaotropic solution in combination. This method is also described by the Japanese Patent Laid-open No. 250681/1995 wherein the purification of only DNA from a microbial organism is carried out using two kinds of cartridges.

[0005] When the chaotropic ion method was used for biological samples such as blood, cells and biological tissues as subjects, DNAs comprised in these biological samples could never be isolated without preliminary treatment of the biological samples, differing from the case of microorganisms. When carrying out the isolation of DNA comprised in blood, proteins are also captured on the carrier by the chaotropic ion method, so that the DNA cannot be efficiently isolated or recovered.

[0006] A known method for DNA isolation from such biological samples different from the chaotropic ion method is that using cationic surfactants.

[0007] A method for precipitating DNA comprised in blood in the presence of 0.5 to 0.6 M sodium chloride and a cationic surfactant alkylbenzyltrimethylammonium salt is disclosed in (U.S. Patent No. 5,010,183).

[0008] Using this method, however, DNA cannot be recovered, when blood is used as the biological sample without any preliminary treatment (separation) or the like. The method requires preliminary treatment, for

example leukocyte separation from blood. Furthermore, DNA yield or purity is not high.

[0009] It is an object of the present invention to provide a method for recovering purified DNA at a high DNA yield, using a biological sample without preliminary treatment.

[0010] It is an additional object of the invention to provide a method for recovering purified DNA at a high DNA yield, using a biological sample without preliminary treatment, wherein the method does not require complicated procedures such as centrifugation or extraction but requires the use of apparatuses of simpler structures and less procedures, so that the method can be automated.

### Summary of the Invention

[0011] The invention relates to a method (first method) for isolating DNA comprised in a biological sample, comprising

- (a) a step of putting a lysing solution, comprising a DNA-containing biological sample, a salt, and a cationic surfactant, and having a salt concentration the same or higher than the concentration initiating the inhibition of DNA precipitation (hereinafter referred to as precipitation inhibition-initiating concentration) into contact with a DNA-binding carrier to allow the DNA comprised in the biological sample to bind to the DNA-binding carrier;
- (b) a step of separating the DNA-bound carrier from other components;
- (c) a step of dissociating the bound DNA from the separated carrier; and
- (d) a step of recovering the dissociated DNA.

[0012] Furthermore, the invention relates to a method (second method) for isolating DNA comprised in a biological sample, comprising

- (A) a step of supplying a solution, comprising a DNA-containing biological sample, a salt, and a cationic surfactant, and having a salt concentration the same or higher than the precipitation inhibition-initiating concentration, into a column with a DNA-binding carrier arranged on a membrane filter, said membrane having a solution retention potency and a solution permeation potency under aspiration, to allow the DNA in the biological sample to bind to the DNA-binding carrier;
- (B) a step of separating the DNA-bound carrier from other components, by removing the lysing solution under aspiration from the column;
- (C) a step of supplying a DNA-dissociating solution into the column, to dissociate DNA from the carrier; and
- (D) a step of recovering a solution comprising the dissociated DNA, by separating the dissociating

solution under aspiration from the column.

#### Brief Description of the Drawings

[0013]

Fig. 1 is a graph depicting the relation between the salt concentration and absorbance at 260 nm, as observed in a reference example; and

Fig. 2 shows the electrophoresis results observed in Example 2.

#### Preferable Embodiments for Carrying out the Invention DNA isolation method (first method)

[0014] At the step (a), a lysing solution, comprising a DNA-containing biological sample, a salt and a cationic surfactant, and having a salt concentration the same or higher than the precipitation inhibition-initiating concentration is put into contact with a DNA-binding carrier to allow the DNA comprised in the biological sample to bind to the DNA-binding carrier. The incubation time of contact with the DNA-binding carrier is determined appropriately, depending on the composition and amount of the lysing solution and the kind and amount of the DNA-binding carrier, but generally, about 3 to 5 minutes are sufficient for the incubation time. Additionally, the incubation can be carried out without heating, but if necessary under appropriate heating, preferably, under condition such that the denaturation of DNA can be avoided.

[0015] The DNA-containing biological sample may be, for example, blood, a cell or a biological tissue. The cell may be a eucaryotic cell or a bacterial cell. Blood is a biological sample with laborious handleability, so that the isolation of DNA from blood is difficult by conventional methods. According to the method of the invention, DNA can be isolated therefrom. The concentration of a DNA-containing biological sample in the lysing solution can appropriately be determined, depending on the kind of the biological sample, the composition of lysing solution and the kind of the DNA-binding carrier used.

[0016] The cationic surfactant includes, for example, at least one surfactant selected from the group consisting of cetyltrimethylammonium bromide, tetradecyltrimethylammonium bromide, dodecyltrimethylammonium bromide, and cetylpyridinium bromide. However, the cationic surfactant is not limited to these surfactants. Additionally, the concentration of the cationic surfactant is determined, taking into account the critical micelle concentration, which is generally within a range of 0.01 to 10 %.

[0017] The salt may be an inorganic acid salt (for example, chloride, nitrate, sulfate, phosphate, and the like) and an organic acid salt (for example, acetate, citrate and the like). More specifically, the salt includes NaCl and other salts (for example, all salts of Na, K, and

Li, in combination with Cl, Br, acetic acid, and formic acid).

[0018] At the step (a), the salt concentration of the solution comprising a DNA-containing biological sample, a salt, and a cationic surfactant is set up above the DNA precipitation inhibition-initiating concentration. The DNA comprised in the solution can thereby be selectively bound to the DNA-binding carrier. The precipitation inhibition-initiating concentration varies, depending on the kind of salt comprised in the solution; and also depending on the kinds and concentrations of other components, even in case that the same salt is comprised. Therefore, the precipitation inhibition-initiating concentration can appropriately be determined for each salt. In case of sodium chloride, for example, the precipitation inhibition-initiating concentration is about 0.6 M, as described below.

[0019] Taking into account the binding efficiency of DNA comprised in the solution to the DNA-binding carrier, the salt concentration of the solution is preferably between the same and 2-fold concentration of the precipitation inhibition-initiating concentration or the salt concentration for solubilizing the total DNA, whichever is the higher. Like the precipitation inhibition-initiating concentration, the salt concentration for solubilizing the total DNA varies, depending on the kind of the salt comprised in the solution; and also varies depending on the kinds and concentrations of other components, even in case that the same salt is comprised. Therefore, the salt concentration can appropriately be determined for each salt. In case of sodium chloride, for example, the salt concentration for solubilizing the total DNA is about 0.8 M, as described below.

[0020] In case that the biological sample comprises at least linear double-stranded DNA and linear single-stranded DNA, the linear double-stranded DNA selectively binds the DNA-binding carrier at the step (a), and is selectively isolated from the biological sample. In this case, a solution additionally comprising a hydrogen bond-cleaving agent is preferably used as the lysing solution. The hydrogen bond-cleaving agent includes for example urea and formaldehyde. The concentration of urea may be for example 10 % (w/v) or more.

[0021] The DNA-binding carrier includes a mesh filter, beads or a powder, comprising a material selected from the group consisting of glass, silica gel, anion exchange resin, hydroxyapatite and celite.

[0022] At the step (b), the DNA-bound carrier is filtered or centrifuged, and subsequently, the resulting carrier is washed with a rinse solution having a salt concentration higher than the precipitation inhibition-initiating concentration, to separate the DNA-bound carrier from other components. The rinse solution has preferably a salt concentration between the same and 2-fold concentration of the precipitation inhibition-initiating concentration or the salt concentration for solubilizing the total DNA, whichever is the higher. Furthermore, the carrier is preferably washed sequentially in a rinse solu-

tion comprising an aqueous solution comprising a cationic surfactant and in a rinse solution comprising an aqueous solution comprising a volatile organic solvent. The use of the rinse solution comprising an aqueous solution comprising a cationic surfactant enables the removal of impurities from the carrier, and then the use of a rinse solution comprising an aqueous solution comprising a volatile organic solvent enables the removal of the cationic surfactant from the carrier. Herein, the volatile organic solvent is preferably ethanol because denaturation of the DNA bound to the carrier is avoidable and rapid evaporation after washing can be carried out.

[0023] After washing, the carrier can be dried, if necessary, but excess drying occasionally prevents the dissociation of the DNA bound to the carrier.

[0024] The rinse solution and/or the lysing solution is preferably a solution comprising glycerol, in order to avoid the adsorption of the DNA-binding carrier to each other, which causes difficulty in handling. The amount of glycerol to be added is for example within a range of 1 to 50 %.

[0025] At the step (c), the separated carrier is mixed with a lysing solution with the conditional composition for solubilizing DNA, to dissociate the bound DNA from the carrier. The lysing solution with the conditional composition for solubilizing DNA may be, for example, water or heated water, while the temperature of the heated water is a temperature appropriately selected for prompt solubilization of DNA.

[0026] At the step (d), a mixture of the solution comprising the dissociated DNA and the carrier is subjected to solid-liquid separation, to recover the dissociated DNA in the form of a solution. The solid-liquid separation may be, for example, centrifugation or filtration.

#### DNA isolation method (second method)

[0027] At step (A), a solution comprising a DNA-containing biological sample, a salt, and a cationic surfactant having a concentration of the salt higher than the precipitation inhibition-initiating concentration, is supplied to a column with a DNA-binding carrier arranged on a membrane filter, the filter having a solution retention potency and a solution permeation potency under aspiration, to allow the DNA in the biological sample to bind to the DNA-binding carrier.

[0028] The second method of the invention has many steps substantially in common to the first method, except for the use of a column in which the DNA-binding carrier is arranged on the membrane filter, the filter having a solution retention potency and a solution permeation potency under aspiration.

[0029] The above column is satisfactorily a single pipe (tube) with one opening arranged with the membrane filter or a plate of a constant thickness with multiple through-holes arranged therein and with a membrane filter arranged over one opening of each such through-hole. In the latter case, DNA isolation from

multiple samples comprising DNA can be carried out concurrently in multiple columns arranged through a plate, so that various samples can advantageously be treated rapidly.

[0030] The DNA-binding carrier includes a mesh filter, beads or a powder, comprising a material selected from the group consisting of glass, silica gel, anion exchange resin, hydroxyapatite and celite.

[0031] Additionally, the membrane filter has a solution retention potency and a solution permeation potency under aspiration. In other words, the membrane filter has a performance such that the solution can be retained on the membrane filter under no aspiration, while the retained solution is discharged through the membrane filter by aspiration from the side opposite to the side for the retention of the solution. By using such membrane filter, advantageously, the incubation time of the DNA-binding carrier with the DNA-containing solution, the discharge of the solution, the washing and the like can be carried out under control. Particularly because no centrifugation is required for solid-liquid separation, advantageously, the method can readily be automated.

[0032] The incubation time in contact with the DNA-binding carrier can appropriately be determined, depending on the composition and amount of the lysing solution, and the kind and amount of the DNA-binding carrier, but generally, about 3 to 5 minutes are sufficient for the incubation time. Additionally, the incubation can be carried out with no heating, but if necessary can be carried out under appropriate heating, preferably, under condition such that the denaturation of DNA can be avoided.

[0033] The DNA-containing biological sample may be, for example, blood, a cell or a biological tissue. The cell may be a eucaryotic cell or a bacterial cell. Blood is a biological sample with laborious handleability, so that DNA isolation from blood is difficult by conventional methods. According to the method of the invention, DNA can be isolated therefrom. The concentration of a DNA-containing biological sample in the lysing solution can appropriately be determined, depending on the kind of the biological sample, the composition of the lysing solution and the kind of the DNA-binding carrier.

[0034] The cationic surfactant includes, for example, at least one surfactant selected from the group consisting of cetyltrimethylammonium bromide, tetradecyltrimethylammonium bromide, dodecyltrimethylammonium bromide, and cetylpyridinium bromide. However, the cationic surfactant is not limited to these surfactants. Additionally, the concentration of the cationic surfactant is determined, taking into account the critical micelle concentration, which is generally within a range of 0.01 to 10 %.

[0035] The salt may be an inorganic acid salt (for example, chloride, nitrate, sulfate, phosphate and the like) and an organic acid salt (for example, acetate, citrate and the like).

[0036] At the step (A), the salt concentration of the solution comprising a DNA-containing biological sample, a salt, and a cationic surfactant is set up above the DNA precipitation inhibition-initiating concentration. The DNA comprised in the solution can thereby be bound to the DNA-binding carrier selectively. The precipitation inhibition-initiating concentration varies, depending on the kind of the salt comprised in the solution, and also varies depending on the kinds and concentrations of other components, even in case that the same salt is comprised. Therefore, the precipitation inhibition-initiating concentration can appropriately be determined for each salt. In case of sodium chloride, for example, the precipitation inhibition-initiating concentration is about 0.6 M, as described below.

[0037] Taking into account the binding efficiency of DNA comprised in the solution to the DNA-binding carrier, the salt concentration is preferably between the same and 2-fold concentration of the precipitation inhibition-initiating concentration or the salt concentration for solubilizing the total DNA, whichever is the higher. Like the precipitation inhibition-initiating concentration, the salt concentration for solubilizing the total DNA varies, depending on the kind of the salt comprised in the solution; and also varies depending on the kinds and concentrations of other components even in case that the same salt is comprised. Therefore, the salt concentration can appropriately be determined for each salt. In case of sodium chloride, for example, the salt concentration for solubilizing the total DNA is about 0.8 M, as described below.

[0038] In case that the biological sample comprises at least linear double-stranded DNAs and linear single-stranded DNAs, the linear double-stranded DNA selectively binds the DNA-binding carrier at the step (A), and is selectively isolated from the biological sample. In this case, a solution additionally comprising a hydrogen bond-cleaving agent is preferably used as the lysing solution. The hydrogen bond-cleaving agent includes for example urea and formaldehyde. The concentration of urea may be for example 10 % (w/v) or more.

[0039] At the step (B), the DNA-bound carrier is separated from other components, by removing the lysing solution. The DNA-bound carrier is separated by filtration through the membrane filter under aspiration from the column, and the resulting carrier is washed with a rinse solution having a salt concentration higher than the precipitation inhibition-initiating concentration. The rinse solution has preferably a salt concentration between the same and 2-fold concentration of the precipitation inhibition-initiating concentration or the salt concentration for solubilizing the total DNA, whichever is the higher. Furthermore, the carrier is preferably washed sequentially in a rinse solution comprising an aqueous solution comprising a cationic surfactant and in a rinse solution comprising an aqueous solution comprising a volatile organic solvent. The use of the rinse solution comprising an aqueous solution comprising a

cationic surfactant enables the removal of impurities from the carrier, and then the subsequent use of the rinse solution comprising an aqueous solution comprising a volatile organic solvent enables the removal of the cationic surfactant from the carrier. Herein, the volatile organic solvent is preferably ethanol because denaturation of the DNA bound to the carrier is avoidable and rapid evaporation after washing can be carried out.

[0040] After washing, the carrier can be dried, if necessary, but excess drying occasionally prevents the dissociation of DNA bound to the carrier.

[0041] The rinse solution and/or the lysing solution is preferably a solution comprising glycerol, in order to avoid the adsorption of the DNA-binding carrier to each other, which causes difficulty in handling. The amount of glycerol to be added is for example within a range of 1 to 50 %.

[0042] At the step (C), DNA is dissociated from the carrier, by supplying a DNA-dissociating solution to the column. The separated carrier is mixed with a lysing solution with the conditional composition for solubilizing DNA, to dissociate the bound DNA from the carrier. The lysing solution with the conditional composition for solubilizing DNA may be for example water or heated water, while the temperature of the heated water is a temperature appropriately selected for prompt solubilization of DNA.

[0043] At the step (D), a solution comprising the dissociated DNA is recovered, by separating the dissociating solution from the column under aspiration.

[0044] The second method of the invention is advantageous in that the method can be promptly automated, because the separation of the solution from the carrier can be done by filtration under aspiration.

### Examples

[0045] The invention is further described in the following examples.

Reference Example (Measurement of precipitation inhibition-initiating concentration)

[0046] Solution composition

0 to 1.5 M NaCl  
30 mM Tris (pH 8.5)  
15 mM EDTA  
0.5 % CTAB (cetyltrimethylammonium bromide)  
Genomic DNA 50 µg/ml

[0047] Individual solutions with the aforementioned composition at NaCl concentrations increasing in 0.05 M increments from 0 to 1.5 M were prepared; and starting of precipitation was observed. After subsequent centrifugation and supernatant discarding, the resulting precipitate was again dissolved in water; so as to assay the quantity of DNA, the absorbance at 260 nm was

measured. The results are shown in Fig. 1. The concentration at which the precipitation started was defined as precipitation start concentration; and the concentration at which the amount of precipitate was retained constant was defined as the precipitation inhibition-initiating concentration of the solution. The precipitation start concentration of the solution was 0.7 M and the precipitation inhibition-initiating concentration was 0.6 M.

#### Example 1

[0048]

1) 750 µl of lysing solution A with the following composition were added to whole blood of 300 µl (for a microfilter of Eppendorf tube size).

Lysing solution A:

Urea 25 % (w/v)  
CTAB (cetyltrimethylammonium bromide) 0.45 %  
NaCl 0.8 M  
EDTA 15 mM  
Tris pH 7.1, 80 mM  
Glycerol 10 % (v/v)  
Diatomaceous earth (manufactured by Sigma, Co.; acid rinsed and calcined) 1 % (w/v)  
Cellulose (manufactured by Sigma, Co.; alpha-cellulose) 0.3%  
Glycerol advantageously allows the easy manipulation of silica matrix and avoids the packing thereof.

The solution comprises a DNA-binding matrix and was agitated with a magnetic stirrer prior to use. The DNA-binding matrix was dissolved together with inert matrices, so as to allow the prompt resuspension of the DNA-binding matrix per se and thereby enhance the reproducibility of the extraction.

After addition of the lysing solution, the mixture was completely mixed together by inversion or weak agitation. The solubilization reaction can be carried out at room temperature or under mild heating, namely at 37 °C. The length of the incubation was 5 minutes.

2) The mixture solution was transferred on an appropriate filter, which was then subjected to reduced pressure. The transfer of the whole lysing reaction solution on the filter enables the simplification of the manipulation. Finally, the contaminating substances were washed off, so that DNA was retained on the DNA-binding matrix.

3) Rinse solution A of 900 µl having the following composition was added to the filter. The volume 900 µl of the rinse solution A to fill the filter funnel was a sufficient volume to completely wash the

whole filter. Under reduced pressure, the rinse solution was removed. Through this step, the residual contaminants present in the filter were removed. This step can optionally be repeated. The reproducibility of the manipulation can thereby be enhanced.

Rinse solution A:

Urea 25 %  
CTAB 0.45 %  
NaCl 0.55 M  
EDTA 15 mM  
Tris pH 7.1, 80 mM  
Glycerol 10 % (v/v)

4) After the removal of the liquid phase, 900 µl of rinse solution B were added to the filter. The cationic detergent can be removed from the filter and from the DNA-binding resin, by using the rinse solution B (alcoholic saline). After the addition of the solution the incubation for several minutes (2 to 4 minutes) was carried out, so as to promote complete ion exchange prior to application of reduced pressure. This step can be repeated, optionally, so as to raise the reproducibility of the manipulation. A reduced pressure was applied until the rinse solution was removed. Optionally, the filter was further washed with 70 % ethanol, satisfactorily, so as to totally remove the trace amount of the salts contaminating in the rinse solution B.

Additionally 900 µl of the rinse solution B were added, followed by incubation at room temperature for 4 minutes. The rinse solution was removed by re-application of reduced pressure.

Rinse solution B:

Triton X100, 0.069 %  
Tris, pH 8.5, 300 mM  
EDTA 7.5 mM  
NaCl 600 mM  
Sodium acetate 600 mM  
Glycerol 30 % (v/v)

(A 2-fold volume of ethanol was added to the resulting solution prior to use, to adjust the solution to a final ethanol concentration of 66 %.)

5) The filter comprising the biological sample was dried under reduced pressure and in aeration for 3 to 5 minutes, until ethanol was removed (optionally, instead of this step, centrifugation process was satisfactorily carried out).

6) 50 to 100 µl of water (preliminarily heated to 60 or 70 °C) were added to the filter. The filter was transferred in a centrifuge tube. The contents were mixed until the DNA-binding matrix turned into fine particles by agitation (this procedure is sometimes an important step); incubated at room temperature for 2 minutes and centrifuged in a micro-centrifugal machine for 30 seconds. The isolated and resus-

pendent DNA can be used as it is.

Total time: 10 to 25 minutes, depending on the application of such optional steps.

## Example 2

[0049] DNA was isolated in the same manner as in Example 1, except for the modification of the NaCl concentration in the lysing solution between 0 and 1.8 M. The aqueous resuspended DNA solution was electrophoresed. The results are shown in Fig. 2. Consequently, it is indicated that DNA was isolated well at a NaCl concentration within a range of 0.6 to 1.0 M. Within a range of 0 to 0.4 M, the resulting DNA was contaminated with the protein in the biological sample, indicating that the purification was insufficient. In case that the NaCl concentration was 1.2 M or more, no DNA could be recovered. This was possibly ascribed to no adsorption of DNA on the carrier.

[0050] In accordance with the invention, a method for recovering purified DNA at a high DNA yield using biological samples without preliminary treatment can be provided.

[0051] The inventive method does not require complicated procedures such as centrifugation or extraction but requires the use of apparatuses of simpler structures and less procedures so that the method can be automated, if necessary.

## Claims

1. A method for isolating DNA contained in a biological sample, comprising

(a) a step of putting a lysing solution, comprising a DNA-containing biological sample, a salt and a cationic surfactant, and having a salt concentration higher than the DNA precipitation inhibition-initiating concentration, into contact with a DNA-binding carrier to allow the DNA in the biological sample to bind to the DNA-binding carrier;

(b) a step of separating the DNA-bound carrier from other components;

(c) a step of dissociating the bound DNA from the separated carrier; and

(d) a step of recovering the dissociated DNA.

2. A method according to claim 1, wherein the salt concentration of the lysing solution is between the same and 2-fold concentration of the DNA precipitation inhibition-initiating concentration or the salt concentration for solubilizing the total DNA, whichever is the higher.

3. A method according to claim 1 or 2, wherein the biological sample is blood, a cell or a biological tis-

sue.

4. A method according to any one of claims 1 to 3, wherein the cationic surfactant comprises at least one surfactant selected from the group consisting of cetyltrimethylammonium bromide, tetradecyltrimethylammonium bromide, dodecyltrimethylammonium bromide, and cetylpyridinium bromide.

5. A method according to any one of claims 1 to 4, wherein the biological sample comprises at least linear double-stranded DNA and linear single-stranded DNA and wherein the linear double-stranded DNA is selectively bound to the DNA-binding carrier at the step (a), and selectively isolated from the biological sample.

6. A method according to claim 5, wherein the lysing solution additionally comprising a hydrogen bond-cleaving agent.

7. A method according to claim 6, wherein the hydrogen bond-cleaving agent is urea or formaldehyde.

8. A method according to any one of claims 1 to 7, wherein the DNA-binding carrier is a mesh filter, beads, a fiber or a powder, comprising a material selected from the group consisting of glass, silica gel, anion exchange resin, hydroxyapatite, celite, cellulose and diatomaceous earth.

9. A method according to any one of claims 1 to 8, wherein at least the step (a) is carried out without heating.

10. A method according to any one of claims 1 to 9, wherein the DNA-bound carrier is filtered or centrifuged at the step (b) and the resulting carrier is washed with a rinse solution having a salt concentration higher than the precipitation inhibition-initiating concentration, to separate the DNA-bound carrier from other components.

11. A method according to claim 10, wherein the rinse solution has a salt concentration between the same and 2-fold concentration of the precipitation inhibition-initiating concentration or the salt concentration for solubilizing the total DNA, whichever is the higher.

12. A method according to claim 10 or 11, wherein the carrier is washed sequentially in a rinse solution comprising an aqueous solution comprising a cationic surfactant and in a rinse solution comprising an aqueous solution comprising a volatile organic solvent.

13. A method according to any one of claims 1 to 12,

wherein the separated carrier is mixed with a lysing solution with the conditional composition for solubilizing DNA at the step (c), to dissociate the bound DNA from the carrier.

14. A method according to any one of claims 10 to 13, wherein the rinse solution and/or the lysing solution comprises glycerol.

15. A method according to any one of claims 1 to 14, wherein a mixture of the solution comprising the dissociated DNA and the carrier is subjected to solid-liquid separation at the step (d), to recover the dissociated DNA in the form of a solution.

16. A method for isolating DNA contained in a biological sample, comprising

(A) a step of supplying a solution comprising a DNA-containing biological sample, a salt, and a cationic surfactant, and having the salt concentration higher than the precipitation inhibition-initiating concentration, to a column with a DNA-binding carrier arranged on a membrane filter, said filter having a solution retention potency and a solution permeation potency under aspiration, to allow the DNA in the biological sample to bind to the DNA-binding carrier;

(B) a step of separating the DNA-bound carrier from other components, by removing the lysing solution under aspiration from the column;

(C) a step of supplying a DNA-dissociating solution to the column, to dissociate DNA from the carrier; and

(D) a step of recovering a solution comprising the dissociated DNA, by separating the dissociating solution under aspiration from the column.

17. A method according to claim 16, wherein the salt concentration of the lysing solution is below 2-fold the precipitation inhibition-initiating concentration or the salt concentration for solubilizing the total DNA, whichever is the higher.

18. A method according to claim 16 or 17, wherein the biological sample is blood, a cell, or a biological tissue.

19. A method according to any one of claims 16 to 18, wherein the cationic surfactant comprises at least one surfactant selected from the group consisting of cetyltrimethylammonium bromide, tetradecyltrimethylammonium bromide, dodecyltrimethylammonium bromide, and cetylpyridinium bromide.

20. A method according to any one of claims 16 to 19,

wherein the biological sample contains at least linear double-stranded DNA and linear single-stranded DNA, and the linear double-stranded DNA is selectively bound to the DNA-binding carrier at the step (A), and selectively isolated from the biological sample.

21. A method according to claim 20, wherein lysing solution additionally comprising a hydrogen bond-cleaving agent if used as lysing agent.

22. A method according to claim 21, wherein the hydrogen bond-cleaving agent is urea or formaldehyde.

23. A method according to any one of claims 16 to 22, wherein the DNA-binding carrier is a mesh filter, beads, a fiber or a powder, comprising a material selected from the group consisting of glass, silica gel, anion exchange resin, hydroxyapatite, celite, cellulose and diatomaceous earth.

24. A method according to any one of claims 16 to 23, wherein at least the step (A) is carried out without heating.

25. A method according to any one of claims 16 to 24, wherein the DNA-bound carrier is filtered at the step (B) and the resulting carrier is washed with a rinse solution having a salt concentration higher than that of the precipitation inhibition-initiating concentration, to separate the DNA-bound carrier from other components.

26. A method according to claim 25, wherein the rinse solution has a salt concentration between the same and 2-fold concentration of the precipitation inhibition-initiating concentration or the salt concentration for solubilizing the total DNA, whichever is the higher.

27. A method according to claim 25 or 26, wherein the carrier is washed sequentially in a rinse solution comprising an aqueous solution comprising a cationic surfactant and in a rinse solution comprising an aqueous solution comprising a volatile organic solvent.

28. A method according to any one of claims 16 to 27, wherein the dissociating solution for use at the step (C) is a solution with the conditional composition for solubilizing DNA.

29. A method according to any one of claims 25 to 28, wherein the rinse solution and/or the lysing solution comprises glycerol.

30. A method according to any one of claims 16 to 29, wherein the concurrent isolation of DNAs from mul-



multiple samples comprising DNA is carried out through multiple columns arranged on a plate.

5

10

15

20

25

30

35

40

45

50

55

Fig.1

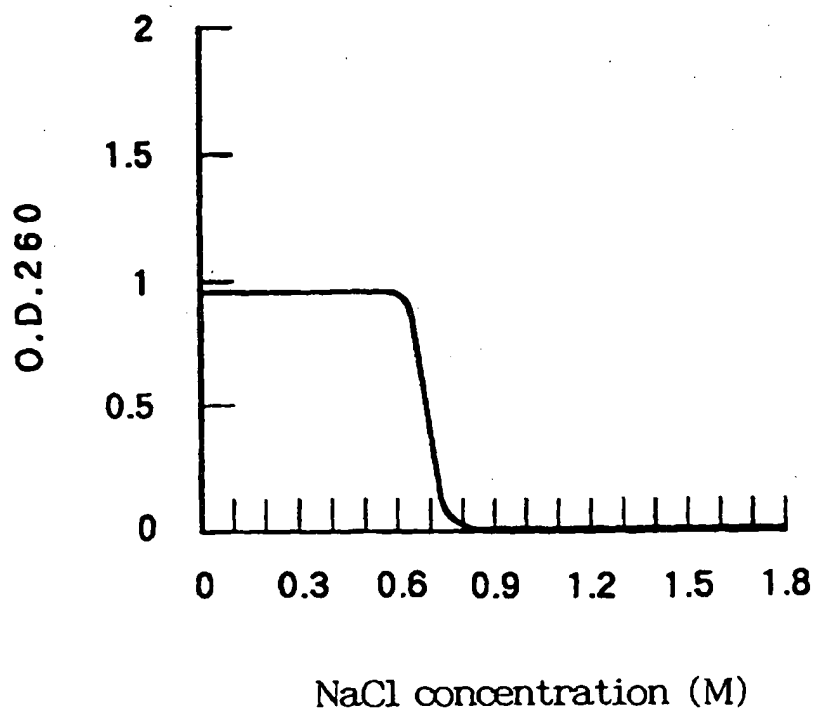
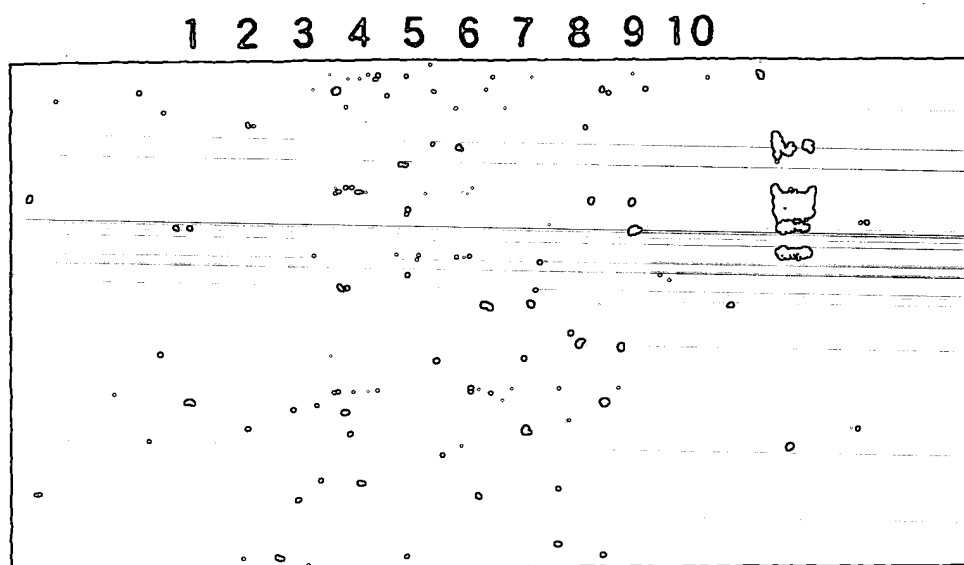


Fig.2



	NaCl concentration (M)
1	0
2	0.2
3	0.4
4	0.6
5	0.8
6	1.0
7	1.2
8	1.4
9	1.6
10	1.8

## INTERNATIONAL SEARCH REPORT

International application No.

PCT/JP98/04201

<b>A. CLASSIFICATION OF SUBJECT MATTER</b> Int.Cl <sup>6</sup> C12N15/10 According to International Patent Classification (IPC) or to both national classification and IPC		
<b>B. FIELDS SEARCHED</b> Minimum documentation searched (classification system followed by classification symbols) Int.Cl <sup>6</sup> C12N15/10, C07H21/04 Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched		
Electronic data base consulted during the international search (name of data base and, where practicable, search terms used) WPI (DIALOG), BIOSYS (DIALOG), MEDLINE (STN), JICST File (JOIS)		
<b>C. DOCUMENTS CONSIDERED TO BE RELEVANT</b>		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	JP, 6-22762, A (Bekton Dickinson Co.), 1 February, 1994 (01. 02. 94) & EP, 555798, A & US, 5342931, A & AU, 9333035, A & CA, 2089119, A	1-30
A	JP, 4-360686, A (Tosoh Corp.), 14 December, 1992 (14. 12. 92) & EP, 517515, A	1-30
A	JP, 5-15373, A (Talent SRL), 26 January, 1993 (26. 01. 93) & EP, 442026, A & AU, 9170296, A & CA, 2019911, A & US, 5596092, A	1-30
A	JP, 2-289596, A (Akzo NV), 29 November, 1990 (29. 11. 90) & EP, 389063, A & NL, 8900725, A & AU, 9052153, A & CA, 2012777, A & ZA, 9002190, A & US, 5234809, A	1-30
<input checked="" type="checkbox"/> Further documents are listed in the continuation of Box C. <input type="checkbox"/> See patent family annex.		
* Special categories of cited documents: "A" document defining the general state of the art which is not considered to be of particular relevance "E" earlier document but published on or after the international filing date "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reasons (as specified) "O" document referring to an oral disclosure, use, exhibition or other means "P" document published prior to the international filing date but later than the priority date claimed "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art "A" document member of the same patent family		
Date of the actual completion of the international search 7 October, 1998 (07. 10. 98)		Date of mailing of the international search report 20 October, 1998 (20. 10. 98)
Name and mailing address of the ISA/ Japanese Patent Office Facsimile No.		Authorized officer Telephone No.

Form PCT/ISA/210 (second sheet) (July 1992)

## INTERNATIONAL SEARCH REPORT

International application No.  
PCT/JP98/04201

## C.(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	Anal. Biochem., Vol. 103[2] (1980) Schneider W.C. et al., "Simplified Isolation and Quantitation of Cytoplasmic DNA from Rat Liver" p.413-418	1-30
A	Biokhimiya, Vol. 42[10] (1977) Naktinis V.J. et al., "Two simple methods for isolation of DNA from various sources using cetavlon" p.1783-1790	1-30
A	Anal. Chem., Vol. 64[22] (1992) Tong X. et al., "Solid-Phase Method for the Purification of DNA Sequencing Recations" p.2672-2677	1-30

Form PCT/ISA/210 (continuation of second sheet) (July 1992)